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Two Novel and Selective Nonimidazole H_3 Receptor Antagonists A-304121 and A-317920: II. In Vivo Behavioral and Neurophysiological Characterization

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ABSTRACT

Pharmacological blockade of central histamine H_3 receptors (H_3Rs) enhances cognition in rodents and offers promise for the clinical treatment of neurological disorders. However, many previously characterized H_3R antagonists are either not selective for H_3Rs or have potentially significant tolerability issues. Here, we present in vivo behavioral and neurophysiological data for two novel and selective H_3R antagonists with improved safety indices. Functional blockade of central H_3Rs was first demonstrated for A-304121 [(4-(3-(4-((2*R*)-2-aminopropanoyl)-1-piperazinyl)propoxy)phenyl)cyclopropylmethanone] (1 mg/kg) and A-317920 [*N*-((1*R*)-2-(4-(3-(4-(cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide] (0.45 mg/kg) by significantly attenuating an acute dipsogenia response to the selective H_3R agonist (*R*)-α-methylhistamine [(*R*)-α-MeHA]. Cognitive performance was improved in a five-trial rat pup avoidance test following administration of A-304121 (10 mg/kg) or A-317920 (3 mg/kg), with efficacy comparable with previously published observations for reference H_3R antagonists thioperamide (10 mg/kg), ciproxifan (3 mg/kg), and GT-2331 [(1*R*,2*R*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole] (1 mg/kg). Social memory was also significantly enhanced in the adult rat with A-304121 (3, 10 mg/kg) and A-317920 (1, 3 mg/kg) at doses that produced no significant change in electroencephalogram slow-wave amplitude activity. Relative therapeutic indices (TIs) of 30 and 42 were estimated for A-304121 and A-317920, respectively, by comparing doses producing adverse effects in general observation studies with potency in inhibitory avoidance, which were superior to TIs of 8, 10, and 18 observed for the reference antagonists thioperamide, ciproxifan, and GT-2331, respectively. A-304121 and A-317920 represent a series of novel, H_3R-selective piperazine amides that enhance cognition in vivo, which could offer advantages over existing H_3R antagonists or cognition-enhancing agents.

Presynaptic histamine H_3Rs are found principally in the central nervous system, functioning as autoreceptors to regulate the release of histamine (Arrang et al., 1983), and as heteroreceptors to modulate release of additional neurotransmitters such as acetylcholine (Bacciottini et al., 2000). Activation of central H_3Rs with the selective agonist (*R*)-α-MeHA inhibits the synaptic release of histamine (Arrang et al., 1987), elicits drinking behavior in the rat and mouse (Clapham and Kilpatrick, 1993; Lecklin et al., 1998; Fox et al., 2002b), and can impair cognition in object recognition and passive avoidance paradigms in the rodent (Blandina et al., 1996). Conversely, significant enhancements in various attentional, learning and memory tasks (Ligneau et al., 1998; Bacciottini et al., 2001; Fox et al., 2002a) have been reported following H_3R blockade such that H_3R antagonists and inverse agonists are under investigation as potential therapeutic agents for the treatment of neurological disorders such as attention deficit hyperactivity disorder and Alzheimer's disease (Onodera et al., 1998; Yates et al., 1999; Passini et al., 2000; Tedford et al., 2000).

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ABBREVIATIONS: H_3R, H_3 receptor; (*R*)-α-MeHA, (*R*)-α-methylhistamine; A-304121, (4-(3-(4-((2*R*)-2-aminopropanoyl)-1-piperazinyl)propoxy)phenyl)cyclopropylmethanone; A-317920, *N*-((1*R*)-2-(4-(3-(4-(cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide; ADHD, attention deficit hyperactivity disorder; GT-2331, (1*R*,2*R*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole; TIs, therapeutic indices; SHR, spontaneously hypertensive rat; RID, ratio of investigation duration; EEG, electroencephalogram; FFT, fast Fourier transform; ANOVA, analysis of variance; ABT-418, (*S*)-3-methyl-5(1-methyl-2-pyrrolidinyl)-isoxazole.

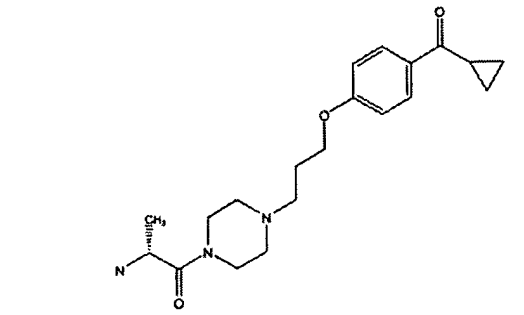
Over the past 15 years, relatively selective H_3R blockers have been identified. These include thioperamide (pK_i for rat H_3 = 8.44, human H_3 = 7.14, human H_4 = 7.32, human α_{2c} = 6.46; Esbenshade et al., 2003), ciproxifan (pK_i for rat H_3 = 9.29, human H_3 = 7.20, human H_4 = 5.73, human α_{2c} = 7.20; Esbenshade et al., 2003), clobenpropit (pK_i for rat H_3 = 9.75, human H_3 = 9.44, human H_4 = 7.38, human α_{2c} = 7.80; Esbenshade et al., 2003), and GT-2331 [(1*R*,2*R*)-4-(2-(5,5-dimethylhex-1-ynyl)cyclopropyl)imidazole] (pK_i for rat H_3 = 9.60, human H_3 = 8.36, human H_4 = 7.08, human α_{2c} = 7.97; unpublished observations). It is possible that the significant affinities of these compounds for α_{2c} and/or H_4 receptors may confound interpretation of some data or result in unwanted side effects at higher doses. Nevertheless, in vitro tissue slice/synaptosome studies and in vivo microdialysis studies have demonstrated increased release of histamine and/or acetylcholine following application or administration of one or more of these compounds (Prast et al., 1999; Bacciottini et al., 2001; unpublished observations). In behavioral studies, improved cognitive performance has been observed with H_3R antagonists across many studies. For example, ciproxifan enhanced performance in a five-choice serial reaction time test of attention in the adult rat (Ligneau et al., 1998); GT-2227, GT-2331, and ciproxifan enhanced acquisition of multitrial inhibitory avoidance tests of learning in rat pups (Yates et al., 1999; Fox et al., 2002a), thioperamide enhanced recall of a passive avoidance response in adult rats (Giovannini et al., 1999) and senescence-accelerated mice (Meguro et al., 1995), and also improved short-term memory in adult rat tests of novel object recognition (Giovannini et al., 1999), social recognition (Prast et al., 1996), and place recognition (Orsetti et al., 2001, 2002).

However, all of these compounds share the imidazole moiety with the endogenous agonist, histamine, conferring the potential for metabolic interactions and promiscuous receptor binding. To avoid these issues, we have developed non-imidazole compounds with improved selectivity and high affinity for H_3Rs. A-304121 [(4-(3-(4-((2*R*)-2-aminopropanoyl)-1-piperazinyl)propoxy)phenyl)cyclopropylmethanone] (pK_i for rat H_3 = 8.60, human H_3 = 6.12, human H_4 = <5.0, human α_{2c} = 5.54; Esbenshade et al., 2003) (Fig. 1) and A-317920 [*N*-((1*R*)-2-(4-(3-(4-(cyclopropylcarbonyl)phenoxy)propyl)-1-piperazinyl)-1-methyl-2-oxo-ethyl)-2-furamide] (pK_i for rat H_3 = 9.15, human H_3 = 7.03, human H_4 = <5.0, human α_{2c} = <5.0; Esbenshade et al., 2003) (Fig. 1), represent this series of novel piperazine amides, and we now present in vivo behavioral and neurophysiological data for these compounds in both mice and rats. For comparison, we chose the reference H_3R antagonists thioperamide, ciproxifan, GT-2331 (clobenpropit was not evaluated due to poor brain penetration; Mochizuki et al., 1996), and methylphenidate, a stimulant efficacious in the treatment of ADHD.

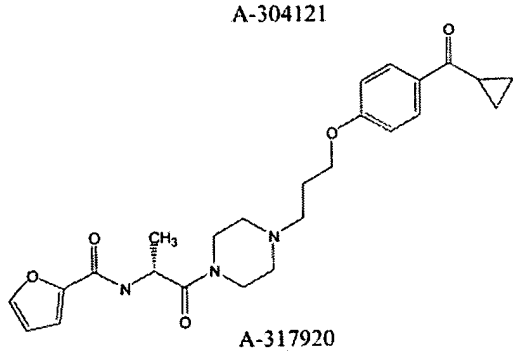
Materials and Methods

Chemicals

Ciproxifan, A-304121, A-317920, and GT-2331 were synthesized at Abbott Laboratories (Abbott Park, IL). Thioperamide and methylphenidate were purchased from Sigma-Aldrich (St. Louis, MO), and (*R*)-α-MeHA was purchased from Tocris Cookson, Inc. (Bristol, UK). Saline (0.9% w/v, Abbott Laboratories) was used as a vehicle, and drug solutions were titrated to pH 6 to 8.



A-304121



A-317920

Fig. 1. Chemical structures of A-304121 and A-317920.

Animals

Mice. Male CD-1 mice were obtained from Charles River Breeding Laboratories (Portage, Canada) at approximately postnatal day 70 (P 70) for dipsogenia studies, at 20- to 25-g body weight for general observation studies, and maintained at Abbott facilities for 10 days before testing. Mice were housed up to 10 per large colony cage (52 cm × 28 cm × 20 cm) in a dedicated quiet room under conditions of 12 h lights on/12 h lights off (on at 6:00 AM), with food and water available ad libitum. Nestlets were provided on cage/bedding change days to reduce territorial fighting. All testing occurred during the light phase.

Rats. Male SHR pups for repeated acquisition avoidance studies were obtained from Harlan (Indianapolis, IN) at postnatal day 7 (P 7) and housed in Abbott facilities until use on days P 20 through P 24 (body weights ranged from 35–50 g). Pups were housed up to 12 per cage (average of two litters) and fostered with Long-Evans lactating females (2 per cage), largely to avoid the poor maternal care of SHR females and possible associated effects on brain and cognitive development (Liu et al., 2000). Adult (350–450 g) and juvenile (75–100g) male Sprague-Dawley rats for social recognition studies and adult (450–550 g) male Sprague-Dawley rats for EEG studies (6 months old) were obtained from Charles River Breeding Laboratories. All rats were housed in a quiet room under conditions of 12 h lights on/12 h lights off (on at 6:00 AM), with food and water available ad libitum. Rats for EEG studies were housed singly. All testing occurred during the light phase.

Surgery. EEG recording electrodes were bilaterally implanted under pentobarbital anesthesia (50 mg/kg, i.p.; Abbott Laboratories) over the parietal cortex (−2.0 mm anterior-posterior, 4.0 mm lateral). A reference electrode was placed 11 mm posterior to bregma and a miniature connector was affixed to the skull. Implanted rats were allowed 2 weeks recovery from the surgery before use. All experiments were conducted in accordance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Dipsogenia Model

Mice were tested in a dipsogenia model as previously described (Fox et al., 2002b). Briefly, each mouse was injected i.p. with either saline, A-304121 (0.3, 1, 10, 30 mg/kg), or A-317920 (0.045, 0.135, 0.45, 1.35, 4.5 mg/kg). After 5 min, saline or (*R*)-α-MeHA (30 mg/kg), was injected i.p. on the opposite side of the abdomen, and the animals returned to their home cages. Following a period of 30 min, mice were then placed separately into smaller cages with access to water via a modified sipper. The mice were left alone for a further 30 min, after which they were removed to their home cages once more and the amount of water consumed was recorded to the nearest 0.01 ml. Sixteen animals were treated at a time for a total of 12 to 16 mice per group.

Five-Trial Inhibitory Avoidance

SHR pups were trained in an inhibitory avoidance response with five acquisition trials as previously described (Fox et al., 2002a). Briefly, the animals were trained to avoid a mild footshock (0.1 mA, 1-s duration) delivered when the pup transferred from a brightly lit to a darkened compartment of a computer-controlled Gemini inhibitory avoidance system (San Diego Instruments, San Diego, CA). After the first trial, the pup was removed and returned to its home cage and littermates, and the transfer latency was noted. One minute later, the same pup was once again placed in the brightly lit compartment, and the training process was repeated for a total of five trials. A criterion time of 1 min applied for the first trial and 3 min for each of the four subsequent trials. Ciproxifan (3 mg/kg, internal positive control), A-304121 (3, 10 mg/kg), A-317920 (3, 10 mg/kg), or saline vehicle was injected s.c. 30 min before the first trial. An oscilloscope (Hitachi V-212, 20 MHz; Hitachi Software Engineering, Yokohama, Japan) and a 100-kOhm resistor were used frequently to ensure correct calibration of the equipment in producing this relatively mild footshock. Pups were not habituated to the avoidance apparatus before the first trial to avoid potentially confounding latent inhibitory effects. Each drug treatment was equally represented among each litter (n = 12/group), and the investigator was normally blinded to treatment. Separate groups of pups ($6 \leq n \leq 12$ per group) were used to control for footshock sensitivity. In these experiments, pups were treated with drug as before, but were exposed to an inescapable footshock that was increased gradually from 0.05 to 0.4 mA and back to 0.05 mA over a period of 30 s. The currents at which vocalization first occurred, termed i_{Max} and then ceased, termed i_{Min}, were noted.

Social Memory

Adult male Sprague-Dawley rats (350–450 g) were separated into fresh investigation test cages and allowed to habituate for 30 min. An unfamiliar juvenile was introduced, and the investigation (grooming, sniffing, close following) duration was recorded over a 5-min period. Both the adult and juvenile were then removed to their respective holding cages. After 90 min, the adult was replaced into the investigation cage and the same juvenile reintroduced 30 min later; investigation duration was again recorded. In a separate group of rats (n = 10), the time interval between the first and second investigation periods was shortened to 30 min to demonstrate inherent social memory of adult rats for juveniles at this earlier time. Ciproxifan (0.1, 0.3, 1, 3 mg/kg), A-304121 (1, 3, 10 mg/kg), A-317920 (0.3, 1, 3 mg/kg), or saline vehicle were administered i.p. to the adult rat immediately after the first exposure period. Immediately after the second investigation period, a new unfamiliar juvenile was introduced to the same adult rat for a further 5 min and the investigation duration recorded. Social memory was quantified by determining, for each adult rat, the ratio of investigation duration (RID) of the second to the first investigation periods. Nonspecific effects were assessed by determining the RID for the unfamiliar juvenile. Group sizes were n = 10 to 16.

Electroencephalogram

EEG (sampling rate 200 Hz) was recorded from previously habituated rats inside sound-attenuating chambers. Before experiments began, a flexible cable was attached to the implanted miniature connector that allowed the rats unrestricted movement during the recording sessions. Standard EEG amplifiers (Grass Instrument Division, Astro-Med, West Warwick, RI) and a computer-based system (Stellate Systems, Montreal, Canada) were used to acquire and analyze data. The average EEG amplitude in microvolts was determined using fast Fourier transform (FFT) analysis and was broken down into an analysis of the 1- to 4-Hz slow-wave band activity.

Dose-response effects on EEG were determined for i.p. administration of A-304121 (3, 10, 30 mg/kg), A-317920 (1, 3, 10 mg/kg), ciproxifan (0.3, 1, 3 mg/kg), and methylphenidate (0.3, 1, 3 mg/kg). The treatments were administered in a random order on different days with one treatment per day and 3 days between each treatment. On one of these treatment days, the rat would receive a vehicle control treatment. This within-subjects design allowed each rat to serve as its own control. EEG recordings were begun within 5 min after injection and recording sessions lasted for 360 min. A total of eight rats were used in these studies.

Spontaneous Locomotor Activity

To assess possible stimulant-like drug effects on spontaneous locomotor activity, adult mice were placed separately into one of 16 acrylic open-field environments (42 cm L × 42 cm B × 40 cm H; Piper Plastics, Copiague, NY) situated inside Versamax/Digiscan monitors, each equipped with 32 horizontal and 16 vertical infrared sensors (AccuScan Instruments, Inc., Columbus, OH) in a darkened room. Mice were allowed to habituate for a period of 30 min and then injected i.p. with drug (methylphenidate, 1, 3, 10 mg/kg; ciproxifan, 1, 3, 10 mg/kg; A-304121, 3, 10, 30 mg/kg; A-317920, 1, 3, 10 mg/kg) or saline vehicle as indicated (n = 12–14 per group) and monitored for 60 min at 1-min intervals. To examine possible inhibitory drug effects on spontaneous locomotor activity, the habituation period was eliminated and adult mice were injected with the same dose of compound (n = 12/group; methylphenidate was not assessed) described above while in their home cages. After 30 min, mice were monitored for an additional 30 min at 1-min intervals using a similar automated activity system (San Diego Instruments) comprising 12 acrylic open-field environments (40 × 40 × 36 cm; Piper Plastics) situated inside 12 monitors, each equipped with 16 horizontal and 16 vertical infrared sensors (San Diego Instruments).

Rotating Rod

To assess possible drug effects on balance, mice from the 30-min locomotor study described immediately above (n = 12/group) were subsequently examined in a rotating rod test at the end of the activity-monitoring period. The apparatus used was an Omnitech Omni Rotor (AccuScan Instruments, Inc.), which consists of four separate rubber-coated rods, 33 mm diameter and 111 mm wide, mounted 365 mm above a wire-grid floor. All four rods are adjacent, but isolated in separate enclosures. Treated mice, one per enclosure, were placed onto a stationary rod, facing away from the front of the apparatus and the investigator. Subsequent depression of the start button activated four timers and a stepper motor that was preprogrammed to increase the rotation rate of all four rods simultaneously from 0 revolutions per min (rpm) to 40 rpm over a 120-s time period. Mice that could no longer keep pace with the rotating rod typically fell to the wire grid, breaking a light beam that halted the corresponding timer. A criterion time of 120 s was used. Each mouse was tested twice in succession, and a mean latency to fall (recorded in seconds) was noted. Latency times for mice who managed to cling to the rotating rod were recorded after two full rotations of the animal on the rod.

General Observation Test

Adult mice were separated into groups of three and placed into observation cages (23 × 21 × 20 cm). Baseline core (rectal) body temperature was recorded with a rapid read digital thermometer (model BAT-12, Physitemp Instruments Inc., Clifton, NJ). In separate experiments, mice were then injected with vehicle, A-304121 (10, 30, 100, 300 mg/kg i.p.), or A-317920 (4, 13, 38, 64, 127, 381 mg/kg i.p.). For comparison, in additional experiments, groups of mice were treated with thioperamide (25, 82, 246 mg/kg i.p.), ciproxifan (3, 10, 30, 100 mg/kg), or GT-2331 (2, 6, 18, 61, 182 mg/kg i.p.). All mice were continuously observed for adverse behavior such as general changes in activity levels, piloerection, ptosis, loss of righting reflex, and seizure activity (including Straub tail, wild running, clonus, tonus) for the first hour and then intermittently at 2, 3, 6, and 24 h after drug administration. All subjective observations (e.g., activity) in drug-treated mice were made with constant reference to a cage of vehicle-treated control mice. Body temperature was recorded 0.25, 0.5, 1, 2, 3, 6, and 24 h following drug administration, and a decrease of 2°C or more was considered hypothermic for the purpose of reporting (Table 1).

Statistical Analyses

Dipsogenia, footshock threshold studies, rotating rod, and social memory data were assessed for significance using a one-way analysis of variance (ANOVA) followed by Tukey's pairwise comparison post hoc tests. Spontaneous locomotor activity data were analyzed using one-way ANOVAs, with time as a repeated measure, followed by Tukey's post hoc tests. Nonparametric Kruskal-Wallis and individual Mann-Whitney *U* tests were used to compare performance in the repeated acquisition avoidance test. For EEG studies, a within-subjects design was used so that each animal served as its own control. A repeated measures one-way ANOVA was used for statistical evaluation of FFT data, with treatment as the repeated measure, and Fisher's post hoc tests for comparisons between treatment groups. A p value <0.05 was considered significant for all post hoc tests. All analyses were performed using Statview 5.0 for Windows (SAS Institute, Inc., Cary, NC).

Results

Dipsogenia Model. (*R*)-α-MeHA (30 mg/kg) induced a pronounced dipsogenia response in vehicle-treated mice, increasing water consumption about 4 to 8 times over basal levels [$F(5,57) = 8.939$, $p < 0.0001$; Fig. 2, A and B]. A-304121 completely blocked dipsogenia induced by (*R*)-α-MeHA in a dose-dependent manner, reaching significance at 1 mg/kg (Fig. 2A). A dose of 30 mg/kg was fully efficacious with respect to vehicle-only controls, and by itself, A-304121 had no effect on basal water intake when tested at 30 mg/kg (Fig. 2A). In a separate experiment, A-317920 also dose-

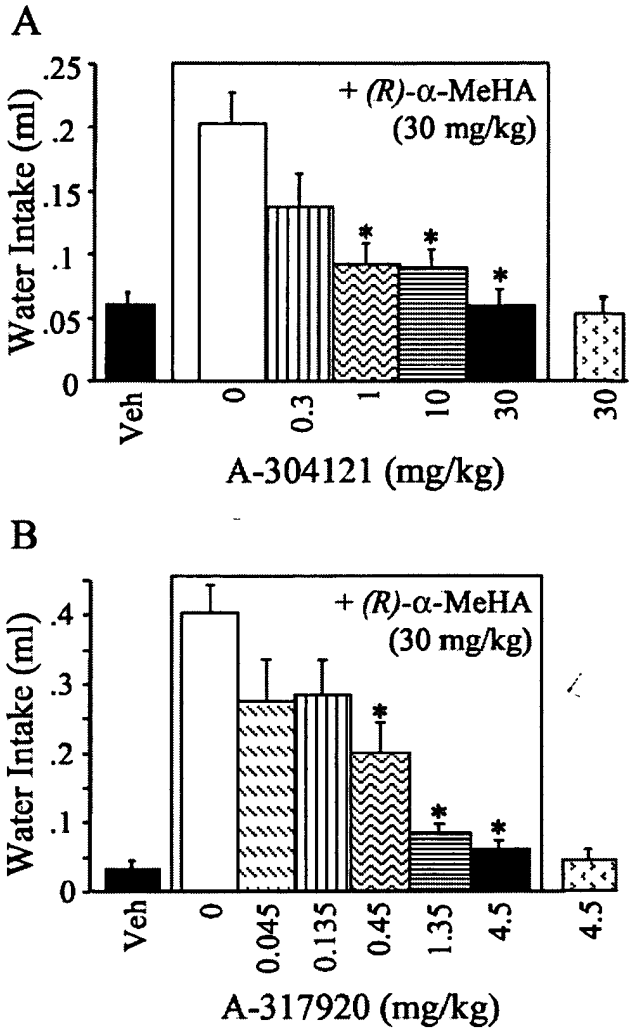


Fig. 2. Attenuation of (*R*)-α-MeHA-induced dipsogenia in mice by A-304121 (A) and A-317920 (B). Mice were dosed with antagonist 5 min before (*R*)-α-MeHA, and water intake was recorded 30 min later for an additional 30-min period. Results represent the mean ± S.E.M. (*, $p <$ 0.05 with respect to 0 + (*R*)-α-MeHA; n = 12–16/group).

TABLE 1

Lowest doses of compounds inducing adverse effect in mice, lowest efficacious doses in rat pup repeated acquisition avoidance, and corresponding therapeutic ratios

Observation	Dose				
	Thioperamide	Ciproxifan	GT-2331	A-304121	A-317920
			mg/kg		
Hypoactivity	82	30	18	100	64
Hypothermia	82	10	18	100	64
Prone posture[a]	N.E.[b]	30	60	300	127
Seizure activity[a]	82	30	18	N.E.[b]	127
Loss righting reflex	N.E.[b]	N.E.[b]	60	N.E.[b]	N.E.[b]
Lethality	246	100	60	N.E.[b]	381
Efficacious dose	10	3	1	10	3
Therapeutic ratio	8	10	18	30	42

N.E., no effect.

[a] The lowest dose at which seizure activity or other pronounced adverse effects were observed (e.g., prone posture, lethality) was used to calculate a therapeutic ratio with respect to efficacious doses in the repeated acquisition inhibitory avoidance model.

[b] No effect observed up to the highest dose tested; for thioperamide, 246 mg/kg; ciproxifan, 100 mg/kg; GT-2331, 182 mg/kg; A-304121, 300 mg/kg; and A-317920, 381 mg/kg.

dependently attenuated the (*R*)-α-MeHA-induced dipsogenia response [$F(8,61) = 14.299$, $p < 0.0001$]; lower doses were required for a statistically significant effect (0.45 mg/kg) and for full efficacy (4.5 mg/kg; Fig. 2B) relative to A-304121 doses.

Repeated Acquisition Avoidance Response. Vehicle-treated SHR pups gradually acquired this task over the five trials, as evidenced by the increase in transfer latencies from the lighted to the dark compartment (Fig. 3, A and B). Treatment of SHR pups with A-304121 did not affect transfer latencies on the first trial, indicating the absence of nonspecific effects on locomotor activity. However, pups treated with A-304121 exhibited prolonged transfer latencies on subsequent trials, indicative of improved acquisition of the task. A Kruskal-Wallis analysis performed across trials 2 through 5 revealed a significant treatment effect ($H = 13.210$, $p = 0.004$). Planned, individual Mann-Whitney comparisons revealed significant ($p < 0.05$) differences between pups treated with A-304121 and those treated with vehicle on trials 3, 4, and 5 (Fig. 3A). Similarly, SHR pups treated with 10 mg/kg A-317920 performed significantly ($p < 0.05$) better than vehicle-treated controls on trials 3, 4, and 5; a significant improvement over controls was also observed on trial 3 for the 3-mg/kg dose (Fig. 3B). Pups in separate internal positive control groups in each experiment that were treated with 3 mg/kg ciproxifan also exhibited an improvement in task acquisition over vehicle-treated controls, showing a significant ($p < 0.05$) effect in trials 3, 4, and 5 for the A-304121 experiment and in trials 3 and 4 for the A-317920 experiment (Fig. 3, A and B). Crucially, footshock sensitivity was not affected by A-304121, ciproxifan [$F(2,15) = 0.538$, $p = 0.594$ for i_{Max}; $F(2,15) = 0$ for i_{Min}; Fig. 4A], or A-317920 [$F(2,15) = 0.661$, $p = 0.531$; $F(2,15) = 0.774$, $p = 0.479$ for i_{Min}; Fig. 4B] as assessed in separate control experiments.

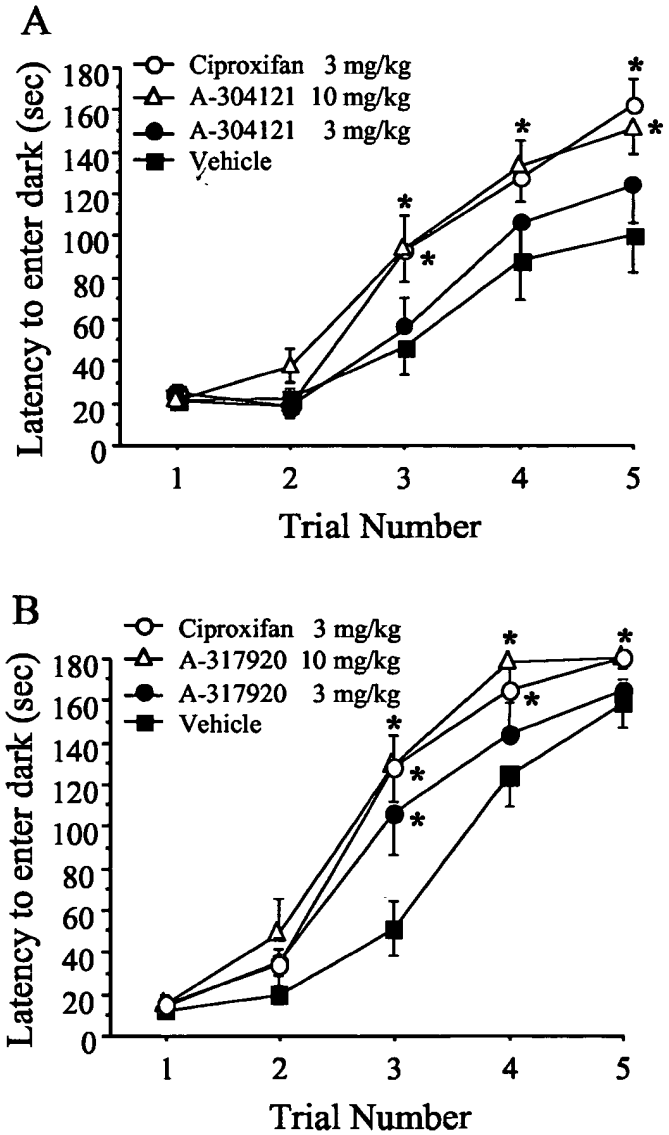


Fig. 3. Enhancement of acquisition of a five-trial inhibitory avoidance response in rat pups with A-304121 (A) and A-317920 (B), with ciproxifan as a positive control in both instances. Rat pups were dosed with antagonist 30 min before the first trial. Data are represented by mean ± S.E.M. for clarity: statistical calculations used nonparametric analyses (*, $p < 0.05$ with respect to vehicle-treated controls; $n = 12$/group).

Social Memory. Thirty minutes after the initial investigation, vehicle-treated adult rats easily recognized the juveniles originally presented in the first investigation period, as evidenced by the decreased duration of investigatory behavior during the second investigation period and the corresponding RID values of around 0.6 (Fig. 5A). In contrast, vehicle-treated adult rats did not remember the juveniles from the first investigation period when allowed to investigate the same juveniles after a 120-min delay, as evidenced by the increased duration of investigatory behavior during the second investigation period and the corresponding RID values of around 1.0 (Fig. 5A), significantly different from the rats with the 30-min interval [$F(1,30) = 9.834$, $p = 0.004$]. No significant differences were observed between groups when a novel juvenile was introduced to the adult immediately after the second trial with the familiar juvenile [$F(1,30) = 1.093$, $p = 0.304$; not shown]. Ciproxifan enhanced social recognition at 120 min, reaching statistical significance at 1 mg/kg [$F(4,75) = 3.081$, $p = 0.021$; Fig. 5B], indicative of positive effects on short-term memory in the adult rat. No significant differences were observed between groups to a novel juvenile introduced immediately after the second trial with the familiar juvenile [$F(4,75) = 1.298$, $p = 0.279$; not shown].

In separate studies, A-304121 [$F(4,45) = 10.924$, $p < 0.0001$] and A-317920 [$F(4,45) = 20.499$, $p < 0.0001$] also enhanced adult rat short-term memory for a familiar juvenile 120 min after administration to the adult of 3 or 10 mg/kg for A-304121 or 1 and 3 mg/kg for A-317920. This effect was reflected by relatively low RID values when compared with vehicle-treated controls (Fig. 6, A and C). In contrast, these same adult rats demonstrated pronounced investigatory behavior for a new juvenile placed into the test cage immediately after the familiar juvenile that did not differ significantly between groups [Fig. 6B, $F(4,45) = 1.490$, $p = 0.221$; Fig. 6D, $F(4,45) = 0.144$, $p = 0.965$], indicating that the decreased investigation was specific for the familiar juvenile, and not due to nonspecific motor, or other, effects of the drugs. In each experiment, positive internal control groups of adult rats that were treated with 1 mg/kg ciproxifan also exhibited an improvement in recall for the familiar juvenile (Fig. 6, A and C), but again investigated extensively a new juvenile placed into the test cage immediately after the familiar juvenile (Fig. 6, B and D).

EEG Slow-Wave Activity. Consistent with its well known central stimulant effects, methylphenidate at 3 mg/kg

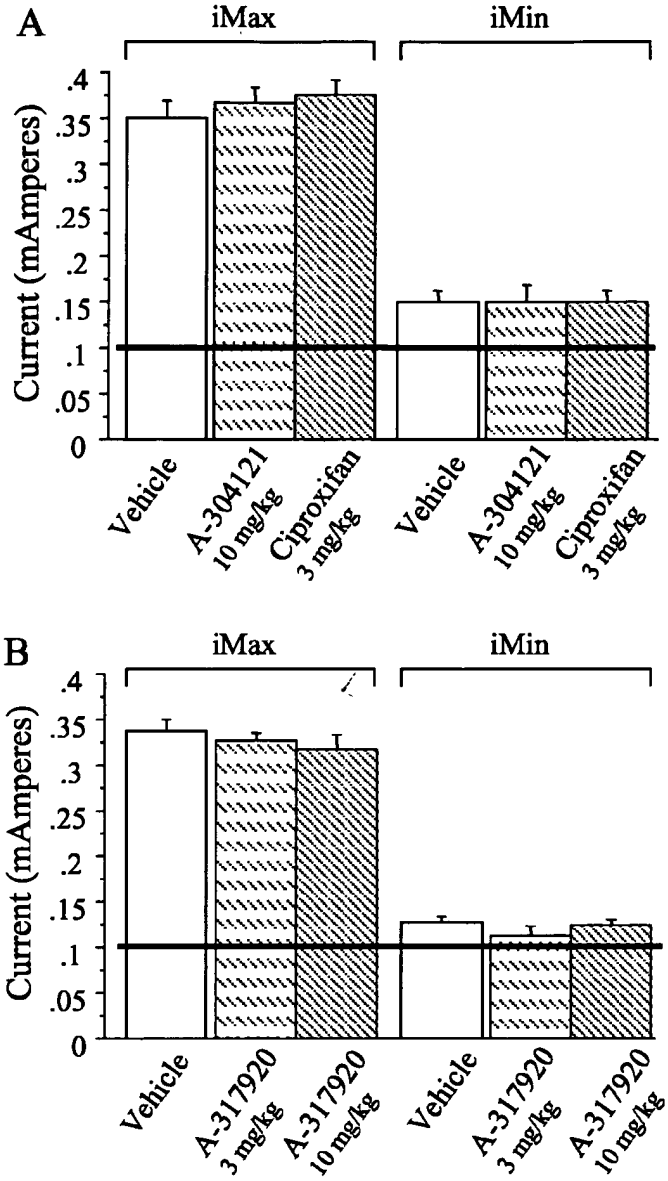


Fig. 4. Lack of effect of A-304121 (A) and A-317920 (B) on sensitivity to footshock, with ciproxifan included for comparison in A. Rat pups were dosed with antagonist 30 min before inescapable exposure to increasing currents (i_{Max}) and subsequent vocalization, followed by decreasing currents (i_{Min}) and subsequent cessation of vocalization. Data are represented by mean ± S.E.M.; n = 6 to 12/group. Horizontal line represents shock level used in learning studies.

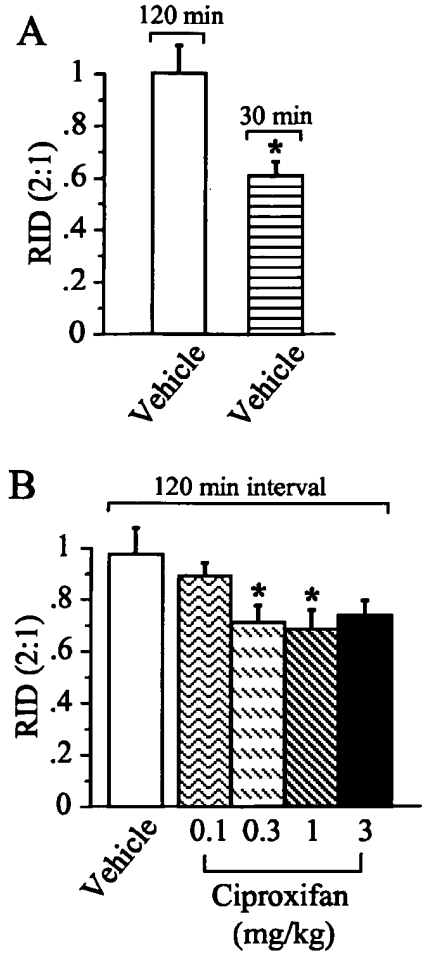


Fig. 5. Social memory in the adult rat. Vehicle-treated rats with either a 30- or 120-min interexposure interval were compared (A). In separate experiments, rats were dosed with antagonist immediately after a 5-min exposure to an unfamiliar juvenile (B). After a period of 120 min, the juvenile was reintroduced to the adult for a second 5-min period and the ratio of investigation between the second and first exposure periods was determined (RID). Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls with 120-min interval; n = 10–16/group).

significantly attenuated slow-wave activity as evidenced by lowered 1- to 4-Hz amplitude when compared with vehicle-treated controls in undisturbed adult rats [$F(3,42) = 8.149$, $p < 0.0001$ for treatment; $F(5,42) = 8.975$, $p < 0.0001$ for time; $F(15,126) = 4.498$, $p < 0.0001$ for treatment × time interaction; Fig. 7A]. However, this effect, which was maximal at 3 mg/kg and is indicative of increased arousal, was short-lived and returned to baseline levels by 3 h following administration. In agreement with previous data (Ligneau et al., 1998), ciproxifan also significantly attenuated slow-wave activity [$F(3,42) = 60.257$, $p < 0.0001$ for treatment; $F(5,42) = 1.258$, $p = 0.300$ for time; $F(15,126) = 2.699$, $p = 0.001$ for treatment × time interaction]; this effect was significant at 1 h after administration for all three doses tested and remained significantly attenuated at 2 h for the higher two doses and up to 4 h for the highest dose of 3 mg/kg (Fig. 7B). In contrast, A-304121 had more modest effects on slow-wave activity [$F(3,42) = 22.871$, $p < 0.0001$ for treatment; $F(5,42) = 0.362$, $p = 0.871$ for time; $F(15,126) = 2.633$, $p = 0.002$ for treatment × time interaction], significantly lowering 1- to 4-Hz amplitude only at the highest dose of 30 mg/kg for up to 2 h following administration (Fig. 7C). Although a significant overall treatment effect was observed for A-317920 [$F(3,30) = 7.913$, $p < 0.0001$], there was no significant treatment × time interaction [$F(15,90) = 0.988$, $p = 0.474$] or time effect [$F(5,30) = 1.135$, $p = 0.364$].

Spontaneous Locomotor Activity and Rotating Rod. Methylphenidate induced a dose-dependent increase in locomotor activity in habituated mice (Fig. 8A), resulting in a significant treatment effect [$F(3,28) = 34.117$, $p < 0.0001$], time effect [($F(59,1652) = 23.114$, $p < 0.0001$] and time × treatment interaction [$F(177,1652) = 6.852$, $p < 0.0001$]. In contrast, no significant increase in locomotor activity was observed for ciproxifan [$F(3,59) = 0.550$, $p = 0.653$ for treat-

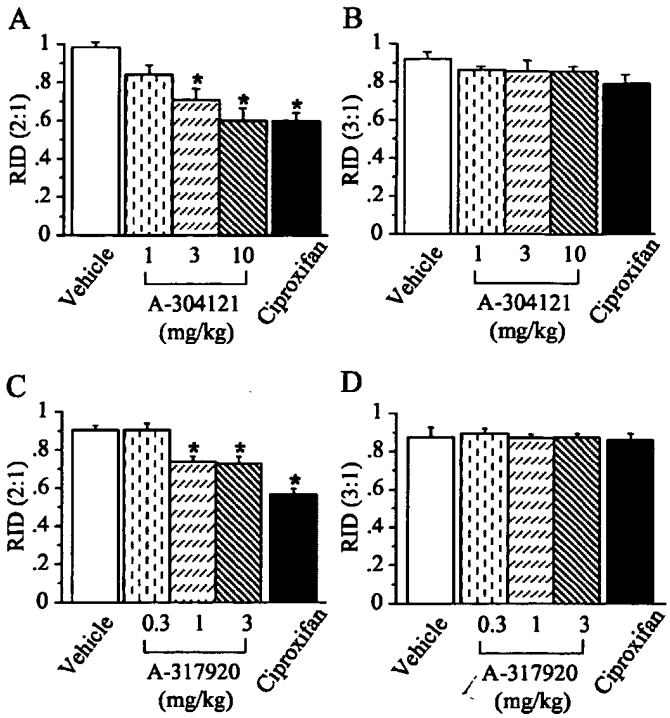


Fig. 6. Enhancement of social memory in the adult rat with A-304121 (A) and A-317920 (C), with ciproxifan (1 mg/kg) as a positive control in both instances. Adult rats were dosed with antagonist immediately after a 5-min exposure to an unfamiliar juvenile. After a period of 120 min, the juvenile was reintroduced to the adult for a second 5-min period, and the ratio of investigation between the second and first exposure periods was determined. Immediately after the second exposure, the adult was exposed for a further 5 min to an additional novel juvenile to control for nonspecific effects of the antagonists (B and D). Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls; n = 10–16/group).

ment], A-304121 [$F(3,59) = 2.028, p = 0.122$ for treatment] or A-317920 [$F(3,59) = 0.092, p = 0.964$ for treatment] over the dose ranges tested (Fig. 8, B–D). Interestingly, ciproxifan, and to a lesser extent A-304121, tended to decrease locomotor activity, particularly at earlier time points (Fig. 8, B and C), although statistical significance was not obtained in this regard.

To further investigate possible hypoactivity effects induced by H_3 receptor antagonists, separate groups of nonhabituated mice were observed. Ciproxifan-treated animals exhibited a significant decrease in locomotor activity [$F(3,29) = 15.087, p < 0.0001$ for treatment; $F(29,1276) = 78.558, p < 0.0001$ for time; $F(87,1276) = 2.398), p < 0.0001$ for treatment × time interaction], particularly at the highest dose (30 mg/kg; Fig. 9, A and D). Mice receiving the highest dose of A-304121 also exhibited significantly decreased locomotor activity ($F(3,29) = 3.552, p = 0.022$ for treatment), although the magnitude of this effect appeared to be less [($F(29,1276) = 86.745, p < 0.0001$ for time; $F(87,1276) = 0.981, p = 0.531$ for treatment × time interaction; Fig. 9, B and E; significance across studies was not compared]. A-317920 was without effect [$F(3,29) = 0.146, p = 0.9316$ for treatment; Fig. 9, C and F], although these data do not preclude an effect under similar conditions at even higher doses (see Table 1 for general observation results at higher doses).

Consistent with these observations, the same mice also exhibited impaired performance in the rotating rod test for ciproxifan [30 mg/kg; $F(3,44) = 2.937, p = 0.044$]. In contrast, A-304121 (100 mg/kg) and A-317920 (30 mg/kg) had no significant effect on rotating rod performance [$F(3,44) = 2.370$, $p = 0.083$ and $F(3,44) = 1.587$, $p = 0.206$, respectively; Fig. 10, A–C].

General Observation Test. Ciproxifan, A-304121, and A-317920 did not induce any observable adverse effects in mice at doses comparable to those effective in dipsogenia, repeated acquisition, or social recognition models (Table 1). Ciproxifan-treated mice were hypothermic and exhibited seizure-like activity at 30 mg/kg, whereas 100 mg/kg induced clonic seizures and was lethal. In contrast, A-304121 did not induce seizure activity at any dose tested, up to a maximum dose of 300 mg/kg, although hypothermia and hypoactivity were observed at this dose. A-317920 caused moderate hypoactivity and hypothermia at 64 mg/kg, seizure activity and pronounced hypothermia at 127 mg/kg, and was lethal at 381 mg/kg. TIs of 10 (ciproxifan), 30 (A-304121), and 42 (A-317920) were calculated for each compound, based on an efficacious dose in the repeated acquisition avoidance model and a dose that induced pronounced adverse effects in the general observation test (Table 1).

Discussion

Modulation of histamine H_3Rs in the mammalian central nervous system has demonstrated important functional roles for these receptors in physiological processes such as food and water intake, learning and memory, and sleep-wake state. However, the bulk of behavioral pharmacology studies published to date with H_3R antagonists and inverse agonists have relied upon imidazole-based compounds having only a modest selectivity for H_3Rs. Thus, therapeutic ratios between efficacious doses and doses inducing serious adverse effects appear low, reducing the possibility for successfully advancing such compounds for clinical applications. We now show that two novel, more selective, non-imidazole H_3R antagonists described in this and a companion article (Esbenshade et al., 2003) have improved behavioral and physiological characteristics compared with reference agents.

Evidence for a functional blockade of central H_3Rs by A-304121 and A-317920 was first established by evaluating both compounds in a previously characterized mouse dipsogenia model (Fox et al., 2002b). Following acute administration of the selective H_3R agonist (*R*)-α-MeHA, a significant increase in acute drinking approximately 4- to 8-fold above basal levels was observed, which was blocked in mice pretreated with A-304121 or A-317920. Neither of the two compounds alone affected basal water intake. Since most evidence points to the dipsogenia response to (*R*)-α-MeHA as being mediated by central H_3Rs (Lecklin et al., 1998; Fox et al., 2002b), the present results support the concept that A-304121 and A-317920 act as functional blockers of central H_3Rs in the mouse.

Potential cognition-enhancing properties of A-304121 and A-317920 were examined in two different behavior tests. First, A-304121 and A-317920 were evaluated in a five-trial, repeated acquisition, inhibitory avoidance task. As previously demonstrated, SHR pups used in this model show a naturally slower learning curve over the five trials when compared with age-, size-, and sex-matched pups from other

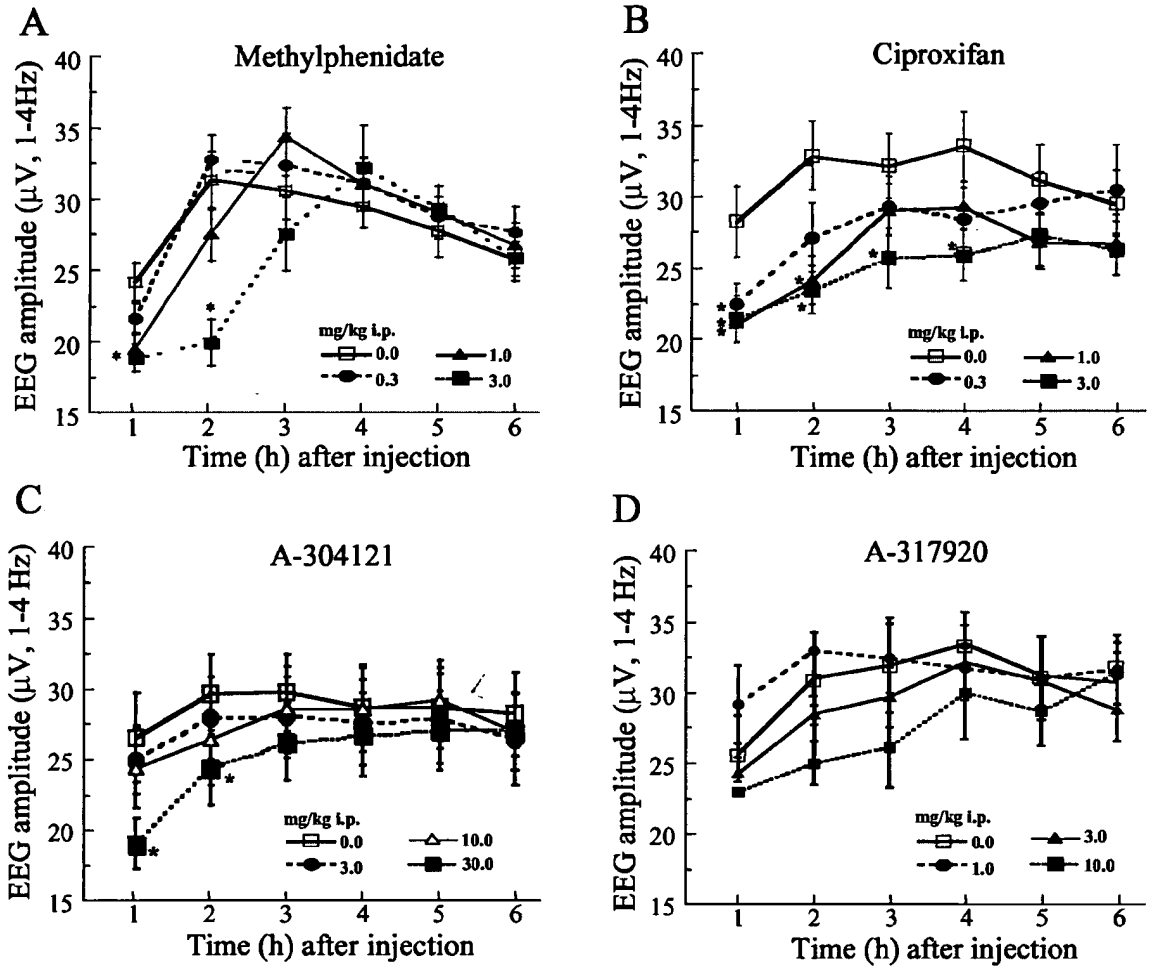


Fig. 7. Effects of methylphenidate (A), ciproxifan (B), A-304121 (C), and A-317920 (D) on slow-wave EEG amplitude in conscious freely moving adult rats. Drugs were administered using a within-subjects design, and EEG recordings commenced 5 min after injection. Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls; total of eight rats).

strains (Fox et al., 2002a). SHR pups administered A-304121 or A-317920 exhibited a trial-dependent and dose-related enhancement of performance compared with vehicle-treated controls. These data compare favorably with pups treated with 3 mg/kg ciproxifan, our standard internal positive control for this model, with methylphenidate (Fox et al., 2002a), a well known treatment for ADHD, and with ABT-418 (Fox et al., 2002a), a nicotinic agonist with efficacy in ADHD in adults. Furthermore, A-304121, A-317920, or ciproxifan at behaviorally relevant doses had no effect on perception of the very mild footshock (0.1 mA, 1 s) used in these studies. SHRs, commonly used as a model for ADHD, have reduced dopaminergic tone, exhibit increased activity and impulsivity in novel surroundings, and display impaired sustained and nonselective attention. The juvenile SHRs (normotensive at this age) used in the present studies, as in our previously published findings (Fox et al., 2002a), appeared to exhibit many of these impaired behaviors in our model. Furthermore, since these impairments are genetic in origin, performance enhancement seen in this model with H_3R antagonists such as A-304121 and A-317920 might be more clinically relevant in treating neurological disorders in which cognitive impairment is a leading characteristic than in other animal models requiring additional pharmacological or surgical intervention.

In a second cognition test, we evaluated the effects of A-304121 and A-317920 on short-term social memory and compared our findings with the effects of ciproxifan. This social recognition model relies on the memory of an adult rat for a juvenile to which the adult rat has been previously exposed. Typically, using olfactory cues, adult rats can remember a previous exposure to a juvenile for approximately 30 to 60 min. However, recall of the adult for the juvenile is usually lost 120 min after the initial exposure. This offers a potential window in which to show enhancement of short-term memory, again without the need for pharmacological or surgical disruption. Adult rats treated with ciproxifan immediately after the first exposure to the juvenile demonstrated enhanced recall for the juvenile when assessed 120 min later. Statistically significant enhanced recall values for ciproxifan-treated rats evaluated at 120 min were similar to those obtained for vehicle-treated controls evaluated at 30 min. Adult rats treated with A-304121 or A-317920 immediately after the first exposure period also showed enhanced recall. These data compared favorably with rats treated with 1 mg/kg ciproxifan, which was tested routinely as our standard positive internal control. Importantly, none of the H_3R antagonists tested affected exploration time of the respective adult rats for unfamiliar juveniles to which the adults were exposed

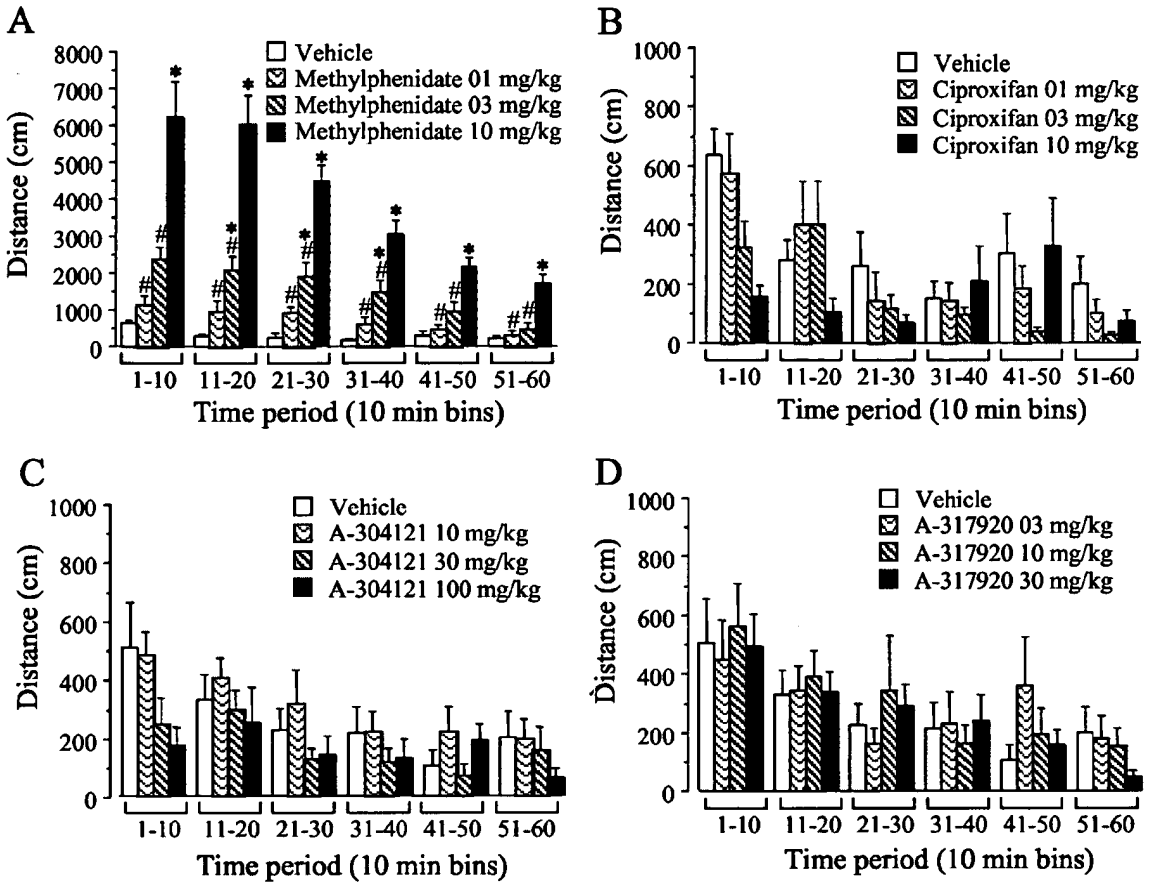


Fig. 8. Effects of methylphenidate (A), ciproxifan (B), A-304121 (C), and A-317920 (D) on spontaneous locomotor activity in mice habituated to the monitoring environment. Drugs were administered 30 min after mice were placed into the activity monitors, and the distance traveled was recorded for a further 60 min. Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls; #, $p < 0.05$ with respect to methylphenidate (10 mg/kg)-treated animals; n = 12–14/group).

immediately after the second exposure to the familiar juvenile, demonstrating that the enhancement of social memory observed is likely not due to nonspecific effects of the compounds on olfactory processing, motivation, or locomotor activity.

Neurophysiological effects of A-304121 and A-317920 were evaluated by assessing slow-wave (1–4 Hz) EEG amplitude for a period of 6 h in conscious, freely moving adult rats with previously implanted surface electrodes. This model was chosen since previously published data from another laboratory indicated that ciproxifan decreased slow-wave amplitude in cats (Ligneau et al., 1998), analogous to a wake-promoting effect. Similarly, the clinical candidate GT-2331, a reported H_3R antagonist, was also shown to have wake-promoting effects in rats using a different paradigm (Tedford et al., 2000). Stimulants such as methylphenidate and amphetamine decrease slow-wave amplitude, also observed in our conscious rat model. In our studies, ciproxifan significantly decreased slow-wave activity at behaviorally relevant doses. These time-dependent effects are consistent with the pharmacokinetic profile for ciproxifan (Ligneau et al., 1998) and similar EEG effects observed in the cat, which have been suggested to contribute to the behavioral efficacy of this compound in various cognition models (Ligneau et al., 1998). However, when rats were treated with A-304121, decreased slow-wave activity was only noted at the highest, supra-efficacious dose, and statistical significance was only observed at 1 and 2 h after administration, despite a half-life in the plasma of 12.9 h for a dose of 10 mg/kg, i.p. in the adult rat (K. Marsh and A. Hancock, unpublished observations). No significant effects on slow-wave activity were observed for A-317920. These results may reflect the improved selectivity of A-304121 and A-317920 over previously described, less selective imidazole-based H_3R antagonists like ciproxifan or GT-2331 and indicate that the decreased slow-wave activity observed with ciproxifan may not be H_3R-mediated. Moreover, given that A-304121 and A-317920 had no effect on EEG slow-wave activity at behaviorally relevant doses, EEG activation, contrary to previous reports, does not appear to be a prerequisite for cognition enhancement.

The potential for stimulant-like activity was further assessed in automated open-field arenas by measuring distance traveled over 60 min in mice that were previously habituated to the arenas for 30 min. As expected, mice treated with methylphenidate exhibited a pronounced and highly significant increase in locomotor activity. In contrast ciproxifan-, and, to a lesser degree, A-304121-treated mice exhibited a tendency toward decreased activity, particularly at higher doses, whereas A-317920 had no effect on locomotor activity, clearly differentiating these compounds from the undesirable stimulant effects of methylphenidate. We also investigated the effect of ciproxifan,

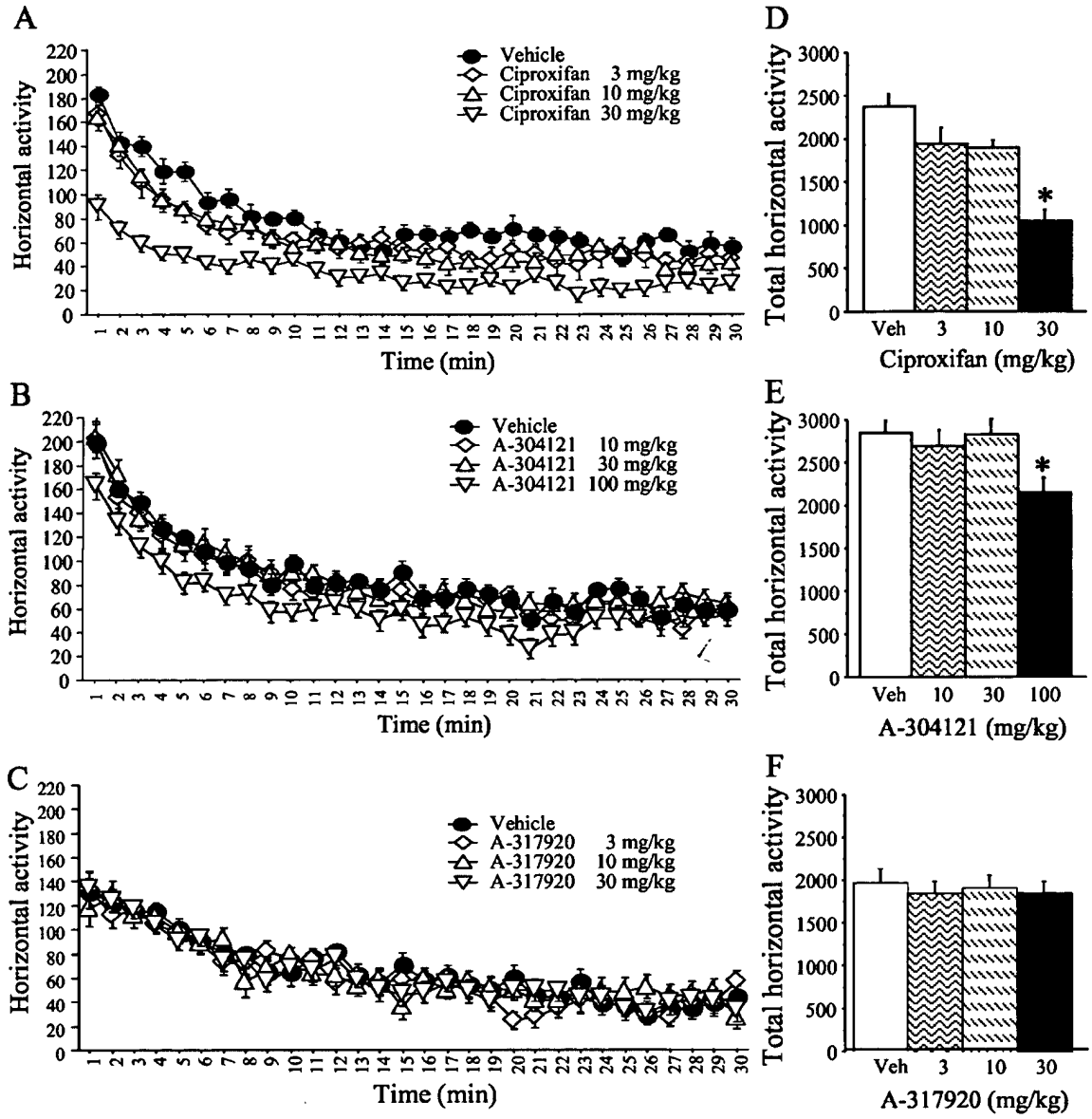


Fig. 9. Effects of ciproxifan (A and D), A-304121 (B and E), and A-317920 (C and F) on spontaneous locomotor activity in nonhabituated mice. Drugs were administered 30 min before mice were placed into the activity monitors and distance traveled was recorded for a further 30 min. Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls; $n = 12$/group).

A-304121, and A-317920 in nonhabituated mice over 30 min. Ciproxifan produced a clear hypolocomotor effect, reaching statistical significance at 10 and 30 mg/kg. A-304121 also induced a less severe hypolocomotor effect at 100 mg/kg, whereas A-317920 was without effect. Similar effects were observed in these same mice when evaluated on a rotating rod.

To identify any additional adverse effects, a general observation test was also conducted in mice receiving one dose from a predetermined range of behaviorally relevant to supramaximal doses of A-304121, A-317920, or for comparison, thioperamide, ciproxifan, or GT-2331. All compounds produced hypoactivity and hypothermia at different doses. GT-2331 uniquely induced a prolonged (at least 6 h) loss of righting reflex accompanied by severe hypothermia that required euthanasia when administered at the relatively high dose of 60 mg/kg. Seizure activity and lethality were observed in all compounds except A-304121. Based on efficacious doses in the cognition models, therapeutic ratios were calculated for each compound with respect to serious adverse effects (typically seizures) in the general observation test. A-304121 and A-317920 demonstrated greater therapeutic ratios than thioperamide, ciproxifan, or GT-2331.

Taken together, the present studies confirm an important role for H_3Rs in regulating cognition. Furthermore, they identify two novel, non-imidazole piperazine amides with functional antagonist activity at central H_3Rs and cognition-enhancing activity at doses well below those inducing adverse effects in locomotor and general observation tests, thus providing overall superior therapeutic ratios when compared with thioperamide, ciproxifan, and GT-2331. For the first time, we also present evidence that activation of EEG patterns is not a prerequisite for cogni-

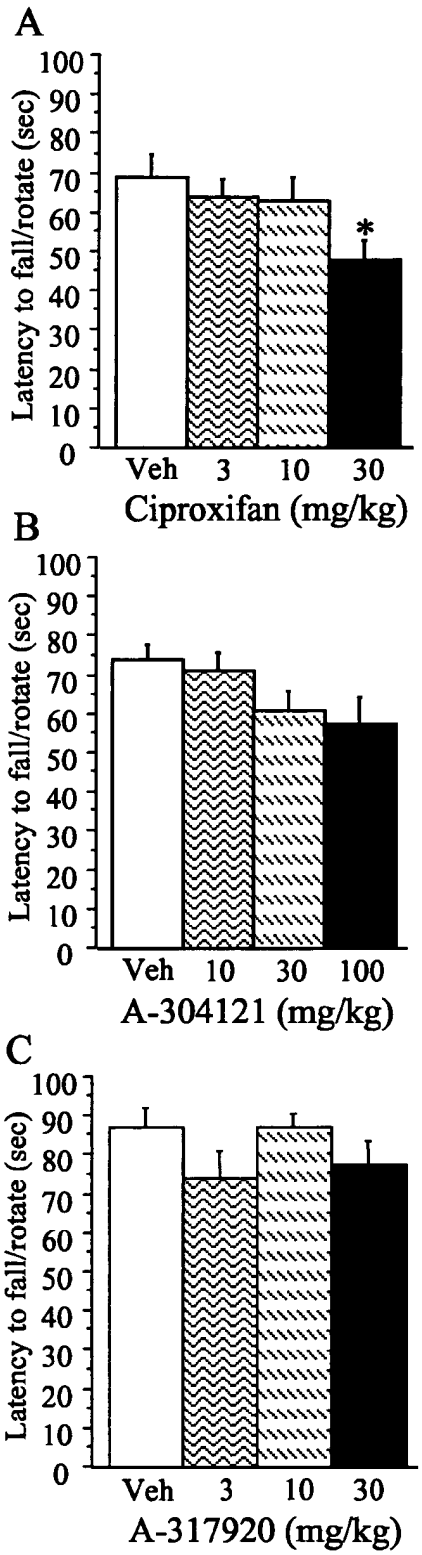


Fig. 10. Effects of ciproxifan (A), A-304121 (B), and A-317920 (C) on latency to fall from a rotating rod. Mice from the locomotor study described in Fig. 9 were removed from the activity boxes and immediately tested on the rotating rod. Data are represented by mean ± S.E.M. (*, $p < 0.05$ with respect to vehicle-treated controls; $n = 12$/group).

tion enhancement with H_3R ligands, further separating the behaviorally beneficial effects of H_3R blockade from the stimulant-like effects of older H_3R antagonists and clinically used stimulants. However, although both A-304121 and A-317920 are highly potent at rat H_3Rs, they exhibit only a comparable (A-317920) or lower (A-304121) affinity for the human H_3R compared with thioperamide, ciproxifan, or GT-2331. Thus, the lower human affinity renders animal pharmacological data relevant to efficacy, but not potency, for A-304121 and A-317920. It would appear that compounds with more balanced affinity profiles for both species may be necessary to improve the likelihood of clinical success.

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References

Arrang J, Garbarg M, Lancelot J, Lecomte J, Pollard H, Robba M, Schunack W, and Schwartz J (1987) Highly potent and selective ligands for histamine H_3 receptors. *Nature (Lond)* **327**:117–123.

Arrang J, Garbarg M, and Schwartz J (1983) Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature (Lond)* **302**:832–837.

Bacciottini L, Giovannelli L, Passani MB, Schunack W, Mannaioni PF, and Blandina P (2000) Ciproxifan and cimetidine modulate c-fos expression in septal neurons and acetylcholine release from hippocampus of freely moving rats. *Inflamm Res* **49**:S41–S42.

Bacciottini L, Passani MB, Mannaioni PF, and Blandina P (2001) Interactions between histaminergic and cholinergic systems in learning and memory. *Behav Brain Res* **124**:183–194.

Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, and Giovannini MG (1996) Inhibition of cortical acetylcholine release and cognitive performance by histamine H_3 receptor activation in rats. *Br J Pharmacol* **119**: 1656–1664.

Clapham J and Kilpatrick GJ (1993) Histamine H_3 receptor-mediated modulation of water consumption in the rat. *Eur J Pharmacol* **232**:99–103.

Esbenshade TA, Krueger KM, Miller TR, Kang CH, Denny LI, Witte DG, Yao BB, Fox GB, Faghih R, Bennani YL, et al. (2003) Two novel and selective non-imidazole histamine H_3 receptor antagonists A-304121 and A-317920: I. In vitro pharmacological effects. *J Pharmacol Exp Ther* **305**:887–896.

Fox GB, Pan JB, Esbenshade TA, Bennani YL, Black LA, Faghih R, Hancock AA, and Decker MW (2002a) Effects of histamine H_3 receptor ligands GT-2331 and ciproxifan in a repeated acquisition avoidance response in the spontaneously hypertensive rat pup. *Behav Brain Res* **131**:151–161.

Fox GB, Pan JB, Esbenshade TA, Bitner RS, Nikkel AL, Miller T, Kang CH, Bennani YL, Black LA, Faghih R, et al. (2002b) Differential in vivo effects of H_3 receptor ligands in a new mouse dipsogenia model. *Pharmacol Biochem Behav* **72**:741–750.

Giovannini MG, Bartolini L, Bacciottini L, Greco L, and Blandina P (1999) Effects of histamine H_3 receptor agonists and antagonists on cognitive performance and scopolamine-induced amnesia. *Behav Brain Res* **104**:147–155.

Lecklin A, Etu-Seppala P, Stark H, and Tuomisto L (1998) Effects of intracerebroventricularly infused histamine and selective H_1, H_2 and H_3 agonists on food and water intake and urine flow in Wistar rats. *Brain Res* **793**:279–288.

Ligneau X, Lin JS, Vanni-Mercier G, Jouvet M, Muir JL, Ganellin CR, Stark H, Elz S, Schunack W, and Schwartz JC (1998) Neurochemical and behavioral effects of ciproxifan, a potent histamine H_3 receptor antagonist. *J Pharmacol Exp Ther* **287**:658–666.

Liu D, Diorio J, Day JC, Francis DD, and Meaney MJ (2000) Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* **3**:799–806.

Meguro K, Yanai K, Sakai N, Sakurai E, Maeyama K, Sasaki H, and Watanabe T (1995) Effects of thioperamide, a histamine H_3 antagonist, on the step-through passive avoidance response and histidine decarboxylase activity in senescence-accelerated mice. *Pharmacol Biochem Behav* **50**:321–325.

Mochizuki T, Jansen FP, Leurs R, Windhorst AD, Yamatodani A, Maeyama K, and Timmerman H (1996) Brain penetration of the histamine H_3 receptor antagonists thioperamide and clobenpropit in rat and mouse, determined with ex vivo [125I]iodophenpropit binding. *Brain Res* **743**:178–183.

Onodera K, Miyazaki S, Imaizumi M, Stark H, and Schunack W (1998) Improvement by FUB 181, a novel histamine H_3-receptor antagonist, of learning and memory in the elevated plus-maze test in mice. *Naunyn-Schmiedeberg's Arch Pharmacol* **357**:508–513.

Orsetti M, Ferretti C, Gamalero R, and Ghi P (2002) Histamine H_3 receptor blockade in the rat nucleus basalis magnocellularis improves place recognition memory. *Psychopharmacology (Berl)* **159**:133–137.

Orsetti M, Ghi P, and Di Carlo G (2001) Histamine H_3 receptor antagonism improves memory retention and reverses the cognitive deficit induced by scopolamine in a two-trial place recognition task. *Behav Brain Res* **124**:235–242.

Passini MB, Bacciottini L, Mannaioni PF, and Blandina P (2000) Central histaminergic system and cognition. *Neurosci Biobehav Rev* **24**:107–113.

Prast H, Argyriou A, and Philippu A (1996) Histaminergic neurons facilitate social memory in rats. *Brain Res* **734**:316–318.

Prast H, Manh Hung T, Fischer H, Kraus M, Lamberti C, Grass K, and Philippu A

(1999) Histaminergic neurons modulate acetylcholine release in the ventral striatum: role of H_3 histamine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **360**:558–564.

Tedford CE, Edgar DM, Sweidel WF, Mignot E, Nishino S, Pawlowski GP, and Yates SL (2000) Effects of a novel, selective and potent histamine H_3 receptor antagonist, GT-2331, on rat sleep-wakefulness and canine cataplexy. *Soc Neurosci Abstr* **26**:460–463.

Yates SL, Phillips JG, Gregory R, Pawlowski GP, Fadnis L, Khan MA, Ali SM, and Tedford CE (1999) Identification and pharmacological characterization of a series of new ^{1}H-4-substituted-imidazoyl histamine H_3 receptor ligands. *J Pharmacol Exp Ther* **289**:1151–1159.

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